Bioaccessibility of heavy metals in seaweeds (*Caulerpa racemosa* var. *corynephora*) : Human health risk from consumption

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Abstract

This research aims to determine the total and bioaccessible concentration of heavy metals (Mn, Fe, Cu, Zn, Cd and Pb) in seaweed (*Caulerpa racemosa var. corynephora*) collected from local markets situated along the Andaman coast of Krabi Province, Thailand. Microwave-assisted acid digestion (EPA Method 3052) was used for sample preparation prior to total metal analysis. The *in vitro* Unified Bioaccessibility Method (UBM) was applied to assess the bioaccessibility of the metals in seaweed samples. The total, gastric phase, and residual fraction concentration were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). The total amounts found in the seaweed samples, in ascending order were: Cd < Pb < Cu < Zn < Mn < Fe with the mean concentration of 0.89, 0.97, 17.4, 59.0, 63.4 and 450 mg/kg dry weight, respectively. High bioaccessibility percentages for Mn (71.8-85.3%) were observed alongside moderate bioaccessibility percentages for Cu (44.3-56.3%), Zn (37.7-47.4%) and Cd (41.8-46.7%), low bioaccessibility percentage for Pb (22.3-32.0%) and a very low bioaccessibility percentage for Fe (11.5-16.5%) were observed. A quality control procedure was implemented which involved analysis of a certified reference material (Seaweed NMIJ CRM 7405-a) for total metal analysis and a mass-balance approach for assessment of the *in vitro* bioaccessibility method.

Keywords: bioaccessibility; heavy metals; ICP-OES; seaweed; risk assessment

1. Introduction

Seaweeds have been consumed since ancient times, particularly in China, Japan and Korea. Nowadays, they have been widely utilized as a valuable resource for human consumption (as fresh, dried, or as a food ingredient) in many countries including America and Europe [1]. It is recognized that seaweeds are an important source of essential minerals, vitamins, dietary fibre, and polyunsaturated fatty acids [2, 3], as well as bioactive compounds, such as, proteins, lipids and polyphenols [4-6].

However, seaweeds may uptake a high content of heavy metals, as their cell wall polysaccharides and proteins contain anionic carboxyl, sulfate and phosphate groups that are excellent binding sites for metal retention [7]. The ability of seaweeds to effectively retain heavy metals is well known [8-14]. The aquatic environments (water temperature, pH, oxygen, salinity, turbidity, nutrient content, and heavy metal contamination) in which seaweeds grow will influence their ability to accumulate heavy metals [15,16]. Many elements, such as, copper, iron, manganese, selenium and zinc present in seaweeds are essential for human physiology, whereas the presence of other elements including arsenic, cadmium and lead, are related to heavy metal contamination, and are considered as toxic to human health [17,18].

In order to estimate the possible effects of heavy metals present in seaweeds, knowledge of the total contents in the sample is not sufficient. It is also important to determine the bioaccessible amount of the metals to humans. Bioaccessibility has been defined as the fraction of a compound that is released from its matrix in the gastrointestinal tract, and thus becomes available for intestinal absorption, i.e. enters the blood stream [19-21]. Several *in vitro* gastrointestinal methods have been developed [22-25] to measure bioaccessibility of heavy metals from environmental samples including food and soil. The approaches are based on extraction techniques that simulate enzymatic action in the compartments of the mouth, stomach and small intestine. The most commonly approaches applied are; the physiologically-based extraction test (PBET), the Dutch National Institute for Public Health and the Environment method (RIVM), the *in vitro* gastrointestinal method (IVG) and the relative bioaccessibility leaching procedure (RBALP) [26-28]. The Bioaccessibility Research Group of Europe (BARGE) is a European network bringing together international institutes and research groups to study human bioaccessibility of priority contaminants in soils such as arsenic, lead, and cadmium via the gastrointestinal tract. BARGE has developed a Unified Bioaccessibility Method (UBM) with the aim of producing a validated and standardized procedure [29]. This method was modified from the RIVM methodology and includes an initial saliva phase and simulated stomach and intestine compartments.

To the best of our knowledge, there is no published information about the heavy metal content and their bioaccessibility in the seaweed (*Caulerpa racemosa* var. *corynephora*) commonly found along the Andaman coast of Krabi Province, Thailand. Hence, the aim of the present study was to determine the total concentrations of heavy metals (Mn, Fe, Cu, Zn, Cd and Pb) in the samples using inductively coupled plasma – optical emission spectrometry (ICP-OES). In addition, the UBM method was applied to the sample and certified reference materials in order to evaluate the bioaccessibility of the heavy metals in the seaweeds. Finally, a risk assessment based on the daily consumption of seaweed is made.

2. Experimental

2.1 Sampling area and sample pretreatment

The seaweed samples were collected from six local markets located near the coast of Krabi province, southern Thailand. The samples which had a high moisture content (97.8%) were oven dried at 60 °C for 48 h and then crushed to a fine powder in an analytical mill (IKA model 11B, Staufen, Germany). All homogenised samples were stored in a dessicator until analysis.

2.2 Chemicals

All solutions were prepared using ultrapure water (18.2 MΩ cm) produced from a Milli-Q™ Millipore Water Purification System (Molsheim, France). All reagents used were of analytical grade. Hydrochloric acid, nitric acid, hydrogen peroxide, sodium dihydrogen phosphate, sodium chloride, sodium sulfate, potassium chloride, ammonium chloride, sodium hydroxide, urea, anhydrous D+glucose, D-glucosaminehydrochloride, and bovine serum albumen were provided by Merck (Darmstadt, Germany), while potassium thiocyante was supplied by Sigma (Shanghai, China) and D-glucuronic acid was obtained from Sigma (St. Gallen, Switzerland). Pepsin, α-amylase, and mucin were obtained from Sigma (Missouri, USA), while uric acid was obtained from Sigma (Budapest, Hungary). Argon gas (99.999%) was supplied by Praxair (Samutprakarn, Thailand). All glass and plastic ware was soaked in a 10% nitric acid bath overnight and rinsed with ultrapure water prior to use. A multi-element standard for manganese, iron, copper, zinc, cadmium, and lead was purchased from AccuStandard, Inc. (Connecticut, USA). The certified reference

material (NMIJ CRM 7405-a, trace elements and arsenic compounds in seaweed) used for method validation was supplied by National Metrology Institute of Japan (Ibaraki, Japan).

2.3 Instrumentation

Heavy metal concentration was measured with an inductively coupled plasma-optical emission spectrometer (ICP-OES, JobinYvon 238 Ultratrace-Ultima2, Longjumeau, France). The instrument was operated under the following conditions: RF power, 1200 W; plasma gas flow rate, 12 L/min; auxiliary gas flow rate, 0.2 L/min; and a sample flow rate, 1.0 ml/min. The elements Cd, Cu, Fe, Mn, Pb and Zn were measured at the following wavelengths 228.802 nm, 324.754 nm, 259.940 nm, 257.610 nm, 220.353 nm and 213.856 nm, respectively. A controlled temperature water bath (Memmert GmbH; Schwabach, Germany) was employed for the *in vitro* bioaccessibility testing. All pH measurements were made with a SevenEasy pH meter (Metler-Toledo AG, Switzerland) which was calibrated daily before use. Acid digestion was carried out using a Multiwave microwave digestion system (Anton Paar GmbH; Graz, Austria) with TFM vessels. The microwave assisted extraction system consists of a magnetron tube (2.45 GHz) giving a maximum power of 1000 W, an oven where the extraction vessels are set upon a turnable, controlling devices for monitoring the temperature and pressure. The microwave system allows up to 12 extraction vessels to be irradiated simultaneously under the maximum pressure of 290 psi and maximum temperature of 300 °C.

2.4 Microwave digestion of samples

The seaweed samples were prepared according to the EPA 3052 standard method [30]. About 0.5 g of sample was accurately weighed into a microwave vessel with 9 mL of 69% HNO_3, 4 mL H_2O, and 0.5 mL of 30% H_2O_2. The mixture was left at room temperature for 30 min to allow easily oxidized material to be digested. The mixture was then extracted at 1000 W under temperature ramp to 100 ºC with 10 min heating time. After cooling, the extracts were filtered through a Whatman No.41 filter paper into a 25 mL volumetric flask, then made up to the mark with ultrapure water and analyzed by ICP-OES. A blank were performed in every digestion batch.

2.5 *In vitro* gastric digestion procedure

The *in vitro* gastric extraction was based on the Unified Bioaccessibility Method (UBM) [29]. Details for the preparation and compositions of simulated saliva and gastric are presented in Broadway et al. [31]. The fluids were prepared one day before carrying out the bioaccessibility extractions to ensure that all of the chemical constituents are thoroughly dissolved. The pH of each fluid was checked and adjusted to the required limits (with 1.0 M NaOH or 37% HCl) i.e. saliva 6.5 ± 0.5 and gastric 1.1 ± 0.1. In addition, the final pH of the mixed saliva and gastric fluid (9.0 mL saliva and 13.5 mL gastric) was checked and adjusted to pH 1.2 ± 0.05. In summary, 0.5 g sub-samples ('gastric' extractions) of seaweed were accurately weighed into a polycarbonate extraction vessel and treated with 9.0 mL of gastric fluid. The mixture was shaken manually (10 s) to ensure thorough mixing and left to stand for 10-15 min. After that, 13.5 mL of gastric fluid was added and the extraction vessels placed in an end-over-end shaker (30 rpm) in a thermostatic water bath maintained at 37 ºC for 1 h. The 'gastric' extraction vessels were removed from the water bath and the pH of the suspension was checked to ensure that it fell within the required pH limit (between pH 1.2-1.5). The 'gastric' extraction solution was centrifuged at 3000 rpm for 15 min and a 5.0 mL aliquot of supernatant removed, made up to 10 mL with 0.1 HNO_3 and stored at <8 ºC prior to determination of the bioaccessible fraction by ICP-OES. The resultant sample residue was further treated by microwave digestion. Gastric extract blanks were performed in every batch.

2.6 Sample analysis by ICP-OES

Extracts of the seaweed samples prepared from both microwave digestion and *in vitro* gastric extraction digests were analyzed by ICP-OES using an external calibration technique. A matrix matched standard solution was used to circumvent potential matrix effects. The quality of the measurement data was evaluated by analyzing the certified reference material (NMIJ CRM 7405-a, trace elements and arsenic compounds in seaweed). The CRMs were run in-between every 10 sample measurements to check the accuracy and precision of the ICP-OES.

2.7 Determination of bioaccessibility

The bioaccessibility measurements are reported as relative bioaccessibility, expressed as a percentage, and calculated according to the following equation;

$$\% \text{ Bioaccessibility} = \frac{C_{\text{bioaccessibility}}}{C_{\text{total}}} \text{ x } 100$$

Where $C_{bioaccessibility}$ is the concentration of heavy metals mobilized from seaweed sample after gastric extraction (mg/kg); and, C_{total} is the total concentration of heavy metals present in seaweed sample after microwave digestion (mg/kg).

3. Results and Discussion

3.1 Analytical features of heavy metal determination

The method validation based on ICP-OES technique was performed by analyzing the certified reference material NMIJ CRM 7405-a, trace elements and arsenic compounds in seaweed). Results showing the accuracy and precision of this method are shown in Table 1. The mean percentage recovery ranged from 92.4 to 104.1 for the elements studied. Good precision (<9.6%), expressed as percentage standard deviation (%RSD), was also obtained. Detection limits were calculated using the expression $3.S_{blank}/m$, where S_{blank} is the standard deviation of 7 replicate measurements of blank and m is the slope of the calibration curve. Typical detection limits were 4.3, 13.5, 1.5, 6.0, 2.1 and 8.6 ng/g for Mn, Fe, Cu, Zn, Cd, and Pb, respectively.

3.2 Total metal determination in seaweed

Total metal concentrations found in seaweed samples collected from 6 sampling locations (S1-S6) after microwave assisted acid digestion and ICP-OES analysis are shown in Table 2. The total metal concentrations found in the samples, in ascending order were: Cd < Pb < Cu < Zn < Mn < Fe with the mean concentration of 0.89, 0.97, 17.4, 59.0, 63.4 and 450 mg/kg dry weight, respectively. Iron was the most abundant metal in the seaweed and the results were similar to the data published by Flores et al. [14] i.e. the Fe concentrations found in Dulse leaf, Nori sheet, Red ogo, and Wakame seaweed was 325, 271, 519, and 382 mg/kg dry weight, respectively. In

contrast, it was relatively high compared to those found in brown seaweed and red seaweed reported by Domínguez-González et al. [8]. However, it was markedly low compared to the high Fe content (38,104 mg/kg) observed in the red seaweed (*Heterosiphonia* genus) reported by Astorga-España et al. [13]. The abundance of Mn and Zn in the seaweed were almost in the same level, with the mean concentrations ranged from 29.4-87.9 and 26.1- 130.7 mg/kg, respectively. The Mn data were in good agreement with those published by Flores et al. [14] and Astorga-España et al. [13] for several species of seaweed. The Mn concentrations were higher than those found in the red seaweed (Dulse and Nori) reported by Domínguez-González et al. [8], but lower than the concentrations found in Red ogo seaweed reported by Flores et al. [14]. The concentrations of Zn found in the present work corresponded to those reported by Besada et al. [9] for Arame, Seaweed spaghetti, Irish moss, Nori, Algae salad, and Seaweed salad and those published by García-Sartal et al. [12] for Kombu and Nori seaweed. Copper amounts found in the seaweed (4.2-38.9 mg/kg) were considered the least abundant among the essential elements studied in this research. The Cu concentrations were comparable to those reported for some species of brown, red and green seaweed [13]. Other researchers have reported lower concentrations of Cu in several seaweed species compared to this study [9,12,32]. Cadmium and Pb, the only two toxic heavy metals studied, had the mean concentrations ranged from 0.777-0.982 and 0.453-1.276 mg/kg, respectively. The data were in the same interval for both Cd and Pb contents found in brown and red seaweed reported by Domínguez-González et al. [8]. In contrast, Cd and Pb concentrations in the seaweed were relatively higher than those reported by Besada et al. [9]. Much higher levels of Cd and Pb presented in the two species of seaweed (*Porphyr*a and *Laminaria*) from different European and Asian countries have been reported [10].

Table 3 shows the heavy metal concentration in the seaweeds presented in 2 different formats i.e. percentage daily value (for essential elements) and mg/kg wet weight (for toxic elements). For the essential elements, they are presented as the percentage daily value in order to assist consumers in interpreting the nutritional information. The percentage daily values are based on the mineral content in a 5 g serving size of dry seaweed [33] as a percentage of the U.S. FDA Daily Reference Value (DV) [34]. After the nutritional value of the seaweeds as a source of essential

elements was calculated, labelling of the seaweed as good or excellent sources of the essential elements was then established according to the U.S. FDA guidance [35]. A 'good' source means a food contains 10 to 19% of the DV per reference amount customarily consumed (5 g serving size of dry seaweed). An 'excellent' source means a food contains 20% or more of the DV per the reference amount customarily consumed. It was found that the seaweeds collected from 3 sampling location (S1, S2 and S5) were classified as good sources of Mn (12.9-16.4% of the DV) and the seaweeds from 2 sampling locations (S3 and S4) were excellent sources of Mn (20.5-22.0% of the DV). Most of the seaweeds (4 out of 6 sampling locations) were good sources of Fe containing 13.0-16.9% of the DV. However, none of the seaweeds were good or excellent sources of Cu and Zn. For toxic elements, the total concentrations on dry weight basis are converted to wet weight in order to compare with the maximum permitted concentrations set by international/national standards. It is clearly observed that the heavy metal concentrations in all seaweed samples were well below the maximum permitted level set by the European Union [36] and the Ministry of Health Thailand [37].

3.3 Oral bioaccessibility of heavy metals from seaweed samples

In order to assess oral bioaccessibility of heavy metals in the seaweeds, the samples were extracted using the *in vitro* gastric extraction procedure. After the extraction, all extracts (gastric and residual) were determined for total metal concentration by ICP-OES. Fig. 1 (A-F) shows bioaccessible contents compared to total metal contents present in the seaweeds for Mn, Fe, Cu, Zn, Cd , and Pb, respectively.

Manganese

The bioaccessible amounts of Mn in the seaweeds were in the range of 13.6 to 49.8 mg/kg dry weight which are relatively high compared to the total concentrations present in the samples (29.4-87.9 mg/kg dry weight) as shown in Fig.1. Mn is considered the most bioaccessible element in this study. The solubility of Mn in the gastric phase were 71.8-85.3% of the total Mn (Fig. 2). From the nutritional information of the seaweeds as a source of Mn, they are considered as 'good' and 'excellent' sources as mentioned in the previous section. Hence, the results from both

the total metal determination and the oral bioaccessibility study suggested that the seaweeds can be recommended for consumers as Mn supplements. The information on bioaccessibility of heavy metals in seaweeds in the literature is quite scarce. A group of researchers in Spain have reported the bioaccessibility of trace elements in different types of edible seaweeds i.e. Wakame, Kombu, Dulse, Sea Spaghetti, Sea lettuce and Nori. The high Mn bioaccessibility (62.3-100%) in the seaweeds was observed which is in agreement to this research. In contrast, low bioaccessibility of Mn were found in cooked seaweed (canned in brine, 18.2%) and a derived product (Agar-Agar, 12.0%) and microalgae (Spirulina, 15.6%). The authors explained that the major dietary fibre (about 90%) in seaweed is soluble fibre enhancing the mineral bioaccessibility in the uncooked seaweeds which contain high fibre [38, 39]. Laird, Chan (2013) have reported high bioaccessibility of Mn (86%) in seaweed (Laver) similar to this research [32].

Iron

The bioaccessible concentration of Fe in the seaweeds were 37.9 to 90.0 mg/kg dry weight which are significantly low compared to the total Fe concentrations (246.7-606.8 mg/kg dry weight) as shown in Fig.1. The bioaccessible fraction of Fe in the gastric phase were 11.5-16.5% (Fig. 2). It is clearly that Fe bioaccessibility is the lowest among all metal studied. From the total metal determination study presented above suggested that most of the seaweeds were good sources of Fe. However, the data from bioaccessibility study do not support the conclusion because Fe was not easily solubilized for absorption. Low bioaccessible fraction of Fe were obtained in the previous report for different kinds of seaweed i.e Dulse (26.9%), Nori (18.9%), Wakame (15.8%), Sea spaghetti (17.9%), Sea lettuce (10.6%) and Canned seaweed (27.8%) [39], which was corresponded to the results of this study. In contast, bioaccessible fraction of Fe in the cooked seaweeds (Kombu, Wakame, Sea lettuce, and Nori) lower than the detection limit was reported [12]. The authors indicated that this is resulted from Fe released into the cooking water during the cooking process.

Copper

Copper considered the second most bioaccessible among all the metals studied with the bioaccessibility ranged from 44.3-56.3 % (Fig.2). The bioaccessible amounts

of Cu in the seaweeds were 1.6 to 15.6 mg/kg dry weight which are moderate compared to the total concentrations present in the samples (4.2-38.9 mg/kg dry weight). Relatively high bioaccessiblity of Cu were found in the seaweeds i.e. Nori (59.5), Kombu (66.5%), Wakame (75.8%), Sea spaghetti (62.5%), Sea lettuce (67.2%) [39]. Laird, Chan (2013) have also reported relatively high bioaccessibility of Cu (59%) in seaweed (Laver) [32].

Zinc

The bioaccessible concentrations of Zn in the seaweeds were 11.2 to 55.0 mg/kg dry weight and the total Zn concentrations present in the samples were 26.1-130.7 mg/kg dry weight (Fig.1). Zinc solubility in gastric phase were 37.7-47.4% (Fig.2). The bioaccessiblity of Zn in the seaweeds is considered moderate. Moreda-Piñeiro et al. (2012) have reported moderate bioaccessiblity of Zn in the seaweeds i.e. Nori (59.1), Kombu (49.9%), Wakame (37.8%), Sea spaghetti (64.5%), and Sea lettuce (41.8%) [39].

Cadmium

The bioaccessible amounts of Cd in the seaweeds were in the range of 0.368 to 0.397 mg/kg dry weight which are moderate compared to the total concentrations present in the samples (0.777-0.982 mg/kg dry weight) as shown in Fig.1. Cadmium bioaccessibility in the gastric phase were 41.8-46.7 % of the total Cd (Fig. 2). For other seaweed species, variations in bioaccessibility of Cd have been observed i.e. Dulse (77.5%), Nori (56.1%), Kombu (78.9%), Wakame (100.2%), Sea Spaghetti (66.7%), Sea lettuce (64.7%), and Canned seaweed (24.0%) [39]. Laird, Chan (2013) have also reported relatively high bioaccessibility of Cd (64%) in seaweed (Laver) [32].

Lead

The bioaccessible concentrations of Pb in the seaweeds were in the range of 0.096 to 0.302 mg/kg dry weight which are relatively low compared to the total concentrations present in the samples (0.453-1.276 mg/kg dry weight) as shown in Fig.1. The bioaccessibility of Pb in the gastric phase were 22.3-32.0% of the total Pb

(Fig. 2). No previously published information is available for Pb bioaccessiblity in seaweed sample.

A mass balance approach was performed in order to evaluate the overall recovery of the *in vitro* gastric extraction method for the six elements. The mass balance validation was made by comparison of the sum of bioaccessible and residual fractions of the CRM against the total metal concentration (Table 1). The validation yielded satisfactory results with percentages of 73.3, 91.6, 92.8, 86.7, 103.8, and 85.4 for Mn, Fe, Cu, Zn, Cd and Pb, respectively. The results indicated that the sample preparation approaches used for the *in vitro* gastric extraction study are reliable and provide accurate results.

3.4 Correlation analysis

The potential influence on the total metal concentrations and bioaccessible concentrations (presented as bioaccessibility) by each element found in the seaweeds was investigated using a correlation analysis. The correlation between the total concentration of a heavy metal and its respective bioaccessibility tended to be non-significant (Mn, Fe, Zn and Pb), except for Cu ($r = 0.770$) and Cd ($r = -0.641$) as shown in Table 4. Significantly negative correlations were observed between the total concentration and the bioaccessibility of Zn and Mn, Cd and Mn, Mn and Fe, Zn and Fe, Fe and Cu, Fe and Zn, Zn and Cu. In contrast, positive correlations were observed between the total concentration and the bioaccessibility of Cu and Fe, Mn and Zn, Cd and Zn. Additionally, significantly positive correlations were observed between the total concentration of Mn and Zn ($r = 0.493$), Zn and Cd ($r = 0.602$), and Cu and Pb ($r = 0.588$). The correlation between the bioaccessibility of Mn and Fe ($r = 0.474$), Fe and Cu ($r = 0.826$) were also significant. García-Sartal et al. (2013) have reported a positive correlation between total Cu concentration and total Fe concentration ($r = 0.779$) in the cooked seaweed [12].

4. Conclusion

The use of the UBM *in vitro* gastric extraction method associated with ICP-OES detection has been shown to be an effective approach to assess the oral bioaccessibility of Mn, Fe, Cu, Zn, Cd and Pb in the seaweeds. High bioaccessibility

percentages for Mn (71.8-85.3%) were observed and moderate bioaccessibility percentages for Cu (44.3-56.3%), Zn (37.7-47.4%) and Cd (41.8-46.7%) were found. A low bioaccessibility percentage for Pb (22.3-32.0%) and a very low bioaccessibility percentages for Fe (11.5-16.5%) were observed. The total metal analysis method was validated by analyzing a certified reference material (Seaweed NMIJ CRM 7405-a); whereas, the procedure of the *in vitro* method was assessed by performing a mass-balance study.

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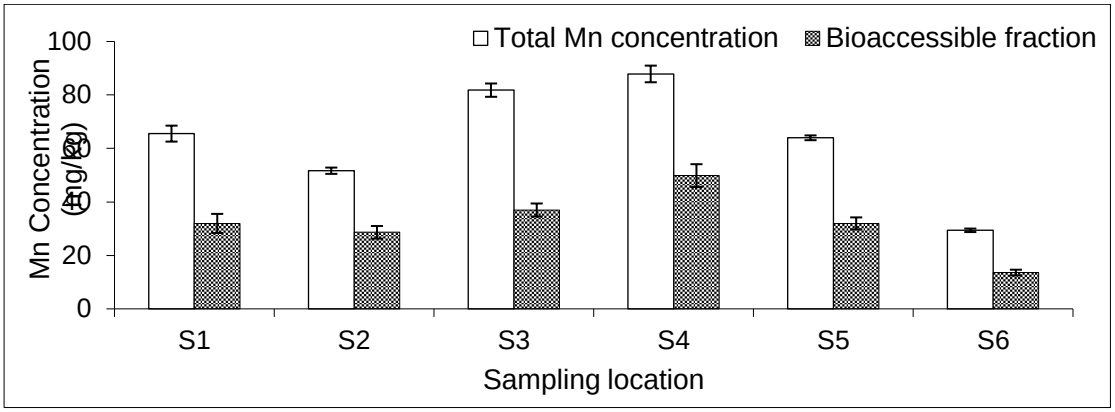
(A)


Total Mn concentration
Bioaccessible fraction
Mn Concentration (mg/kg)
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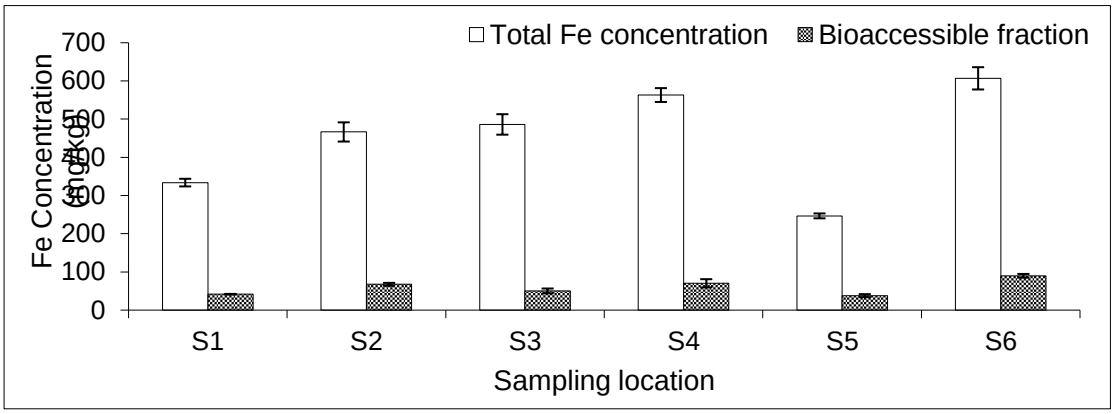
80
60
40
20
0
S1
S2
S3
S4
S5
S6
Sampling location

(B)


Total Fe concentration
Bioaccessible fraction
Fe Concentration (mg/kg)
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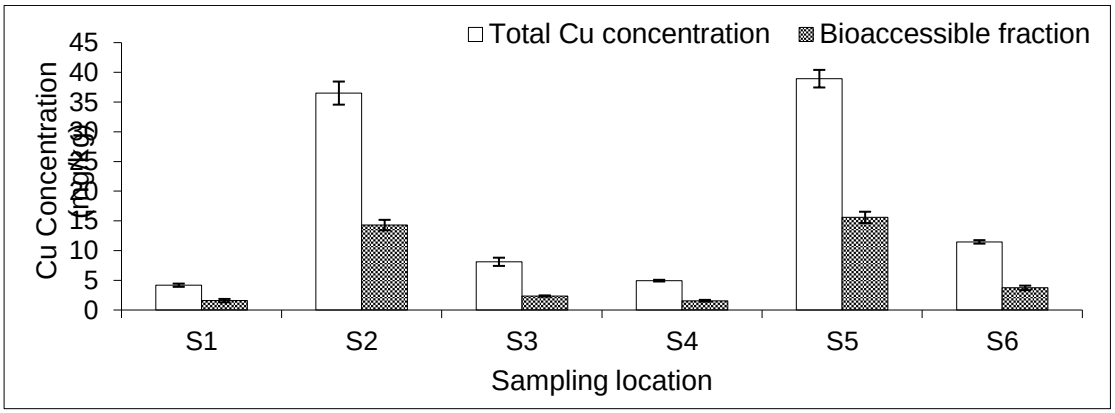
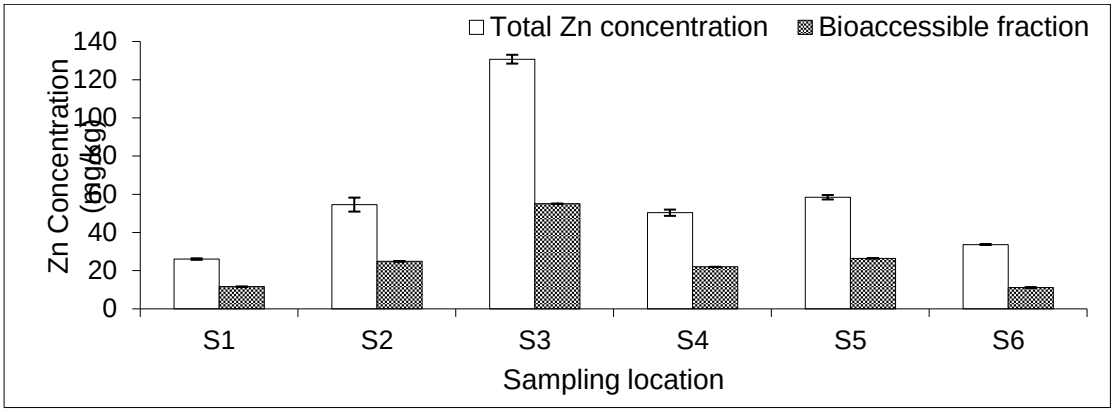
600
500
400
300
200
100
0
S1
S2
S3
S4
S5
S6
Sampling location

(C)


Total Cu concentration
Bioaccessible fraction
Cu Concentration (mg/kg)
45
40
35
30
25
20
15
10
5
0
S1
S2
S3
S4
S5
S6
Sampling location

(D)



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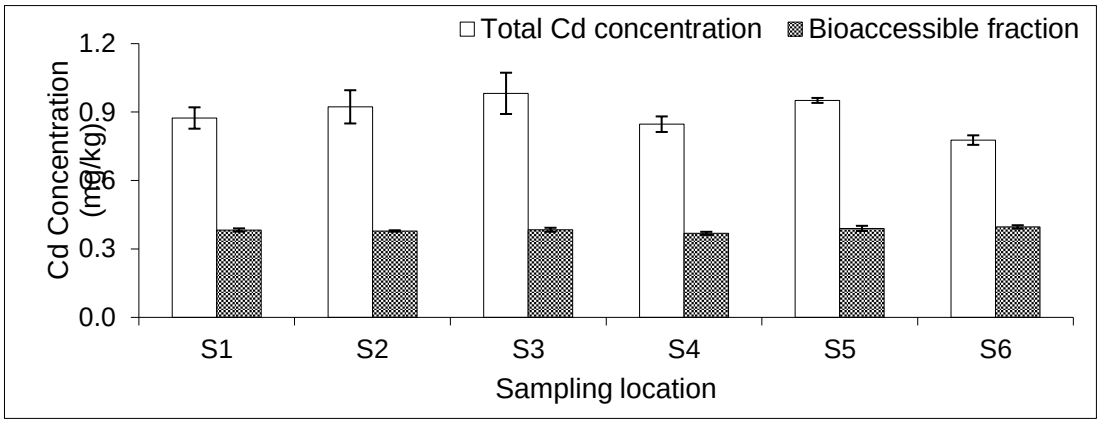

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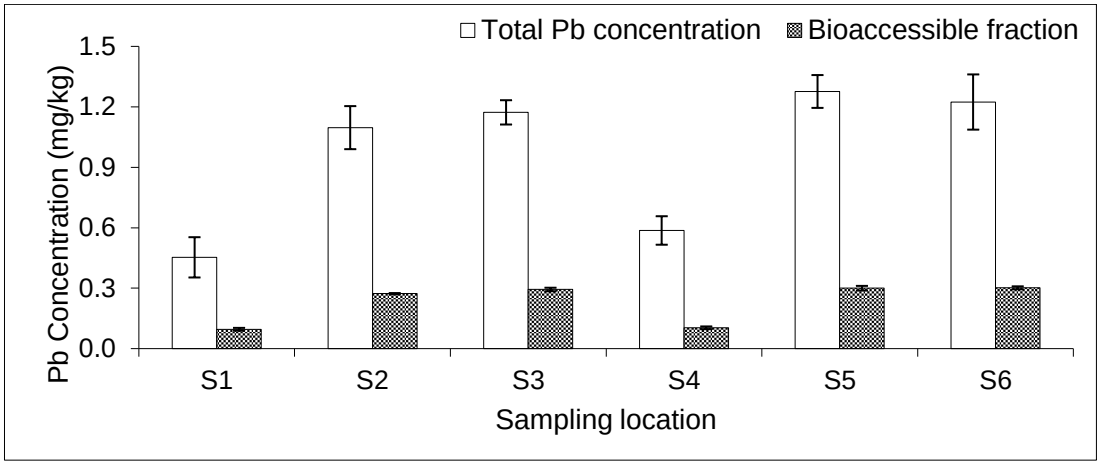

Fig. 1 Bioaccessible and total metal concentrations (mg/kg, dry weight) in seaweed samples

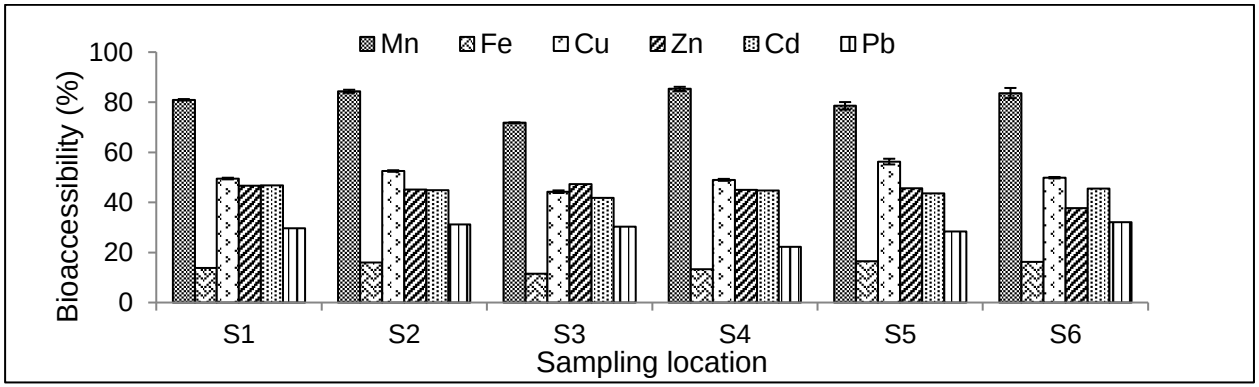

Fig. 2 Percentage bioaccessibility of Mn, Fe, Cu, Zn, Cd and Pb

Table 1. Total and Bioaccessible metal concentrations in NMIJ CRM 7405 (all data reported as dry weight)

Element	Certified values (mg/kg)	Total values		Gastric Phase Mean ± SD (mg/kg)[+]	%BAF (%)	Residue Mean ± SD (mg/kg)[+]	Residue (%)	Σ Gastric + Residue (mg/kg)	Mass balance (%)[#]
		Mean ± SD (mg/kg)* (%)	%RSD						
Mn	14.1 ± 0.7	14.6 ± 0.5 (103.5)	3.2	8.4 ± 0.2	53.9 ± 2.2	2.3 ± 0.4	14.8 ± 2.5	10.7 ± 0.4	73.3
Fe	311 ± 11	324 ± 11 (104.1)	3.3	37.1 ± 1.7	11.7 ± 0.7	257.9±16.7	81.3 ± 2.0	295 ± 17	91.6
Cu	1.55 ± 0.07	1.4 ± 0.1 (92.4)	3.3	0.6 ± 0.1	33.7 ± 4.4	0.7 ± 0.1	44.3 ± 7.9	1.3 ± 0.1	92.8
Zn	13.4 ± 0.5	12.9 ± 0.1 (96.4)	1.6	5.1 ± 1.0	42.7 ± 4.9	5.9 ± 0.3	49.6 ± 4.1	11.1 ± 1.0	86.7
Cd	0.79 ± 0.02	0.77 ± 0.03 (97.9)	9.6	0.38 ± 0.01	45.7 ± 3.2	0.45 ± 0.06	54.3 ± 3.2	0.83 ± 0.06	103.8
Pb	0.43 ± 0.03	0.41 ± 0.05 (96.1)	9.4	0.10 ± 0.02	22.6 ± 3.5	0.25 ± 0.02	55.1 ± 1.7	0.35 ± 0.02	85.4

* N = 7

+ N = 3

[#] Mass Balance = (Σ Gastric + Residue / Total) * 100

Table 2. Heavy metal concentrations in seaweed samples collected from markets in Krabi, southern Thailand

Sampling sites	Mean ± SD (mg/kg, dry weight), n = 3					
	Mn	**Fe**	**Cu**	**Zn**	**Cd**	**Pb**
S1	65.5 ± 3.0	334 ± 10	4.2 ± 0.3	26.1 ± 0.4	0.87 ± 0.05	0.45 ± 0.10
S2	51.7 ± 1.2	467 ± 25	36.5 ± 1.9	54.6 ± 3.7	0.92 ± 0.07	1.10 ± 0.11
S3	81.8 ± 2.5	486 ± 27	8.1 ± 0.7	130.7 ± 2.3	0.98 ± 0.09	1.17 ± 0.06
S4	87.9 ± 3.1	563 ± 18	4.9 ± 0.1	50.3 ± 1.6	0.85 ± 0.03	0.59 ± 0.07
S5	64.0 ± 0.9	247 ± 6	38.9 ± 1.5	58.4 ± 1.2	0.95 ± 0.01	1.28 ± 0.08
S6	29.4 ± 0.7	607 ± 29	11.5 ± 0.3	33.7 ± 0.3	0.78 ± 0.02	1.22 ± 0.14
Mean	63.4	450	17.4	59.0	0.89	0.97

Sampling sites (Latitude and longitude coordinates)

S1 = Ao Luek Tai Municipal Food Market, Ao Luek, Krabi (8°22′35.9″N 98°43′16.5″ E)

S2 = Khao Khram Market, Muang, Krabi (8°14′03.6″N 98°48′31.0″ E)

S3 = Ban Bang Phueng Market, Nuea Khlong, Krabi (8°02′22.1″N 99°05′00.4″ E)

S4 = Rungrot Market, Nuea Khlong,Krabi (8°04′09.1″N 98°59′58.9″ E)

S5 = Khlong Thom Market, Khlong Thom, Krabi (7°56′15.5″N 99°08′37.1″ E)

S6 = Hua Hin Pier, Koh Lanta, Krabi (7°41′35.8″N 99°05′57.1″ E)

Table 3. Heavy metal concentrations in seaweeds expressed as percentage daily value (for essential elements) and mg/kg wet weight (for toxic elements)

Sampling location	Daily value[a] (%) per 5 g dry weight serving				mg/kg wet weight	
	Mn	**Fe**	**Cu**	**Zn**	**Cd**	**Pb**
S1	16.4*	9.3	1.0	0.9	0.019	0.010
S2	12.9*	13.0*	9.1	1.8	0.020	0.024
S3	20.5**	13.5*	2.0	4.4	0.022	0.026
S4	22.0**	15.6*	1.2	1.7	0.019	0.013
S5	16.0*	6.9	9.7	1.9	0.021	0.028
S6	7.3	16.9*	2.9	1.1	0.017	0.027
DV (mg)	2.0	18	2.0	15	NA	NA
Standard limit	NA	NA	NA	NA	1.0[b]	1.5[b], 1.0[c]

* Good source of essential elements based on a serving provided (10-19% DV)

** Excellent source of essential elements based on a serving provided (≥20% DV)

[a] Daily value (%) was calculated as [average metal concentration (mg/5 d dry weight) divided by DV (mg)]x100

[b] Standard limits set by the European Union (Official Journal of the European Union, 2006)

[c] Standard limits set by Ministry of Health Thailand (1986)

NA, Not Applicable

Table 4. Pearson correlation (r) between total concentration and bioaccessibility measured in seaweed samples

	Pseudo-total concentration (mg/kg)						Bioaccessibility (%)					
	Mn	**Fe**	**Cu**	**Zn**	**Cd**	**Pb**	**Mn**	**Fe**	**Cu**	**Zn**	**Cd**	**Pb**
Pseudo-total concentration (mg/kg)												
Mn	1.000	-0.159	-0.300	0.493*	0.458	-0.428	-0.332	-0.730**	-0.347	0.749**	-0.174	-0.455
Fe	-	1.000	-0.467	0.061	-0.459	0.017	0.326	-0.189	-0.546*	-0.545*	-0.061	-0.022
Cu	-	-	1.000	-0.023	0.344	0.588*	0.067	0.663**	0.770**	0.050	-0.260	0.160
Zn	-	-	-	1.000	0.602**	0.411	-0.781**	-0.594**	-0.514*	0.432	-0.358	0.047
Cd	-	-	-	-	1.000	0.230	-0.555*	-0.260	0.024	0.679**	-0.641**	0.179
Pb	-	-	-	-	-	1.000	-0.303	0.372	0.223	-0.313	-0.349	0.282
Bioaccessibility (%)												
Mn	-	-	-	-	-	-	1.000	0.474*	0.387	-0.452	0.132	-0.160
Fe	-	-	-	-	-	-	-	1.000	0.826**	-0.467	-0.054	0.136
Cu	-	-	-	-	-	-	-	-	1.000	-0.057	-0.096	0.074
Zn	-	-	-	-	-	-	-	-	-	1.000	-0.194	-0.144
Cd	-	-	-	-	-	-	-	-	-	-	1.000	-0.145
Pb	-	-	-	-	-	-	-	-	-	-	-	1.000

** P < 0.05*
*** P < 0.01*